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New Electrorelease Systems Based on Microporous Membranes

by

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New Electrorelease Systems Based on Microporous Membranes

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ABSTRACT

Electrorelease systems have previously been based on electrochemical release of molecules chemically entrapped in polymer membranes. A new type of electrorelease system is described; this system is based on a microporous Al_2O_3 membrane covered with a barrier layer which seals the pores. Electrorelease occurs upon electrochemical dissolution or disruption of the barrier layer, which opens the pores. Two different barrier layers are investigated: silver and Na^+-form Nafion. The electrorelease rate can be controlled by partially removing the barrier layer or by dividing the barrier layer into several individually-addressable zones. This configuration may also be used as a multi-dose device.

INTRODUCTION

Electrorelease is the use of electrochemistry to control the delivery of a chemical or drug. A variety of electroreleasing polymers have been described in the literature (1-5). These systems rely on either electrochemical cleavage of the chemical bond holding the reagent to be released to a polymeric support (1), or electrostatic exclusion of an ionic species from an electroactive polyelectrolyte (2-5). We have recently described a new type of electrorelease system based on a novel composite membrane (6). This new system is unique in that it allows release of macromolecules and proteins (e.g. insulin) (6).

This composite membrane electrorelease system consists of a microporous support membrane which is covered with a nonporous barrier layer (6). When the barrier layer is intact, the reagent to be released is entrapped in the microporous membrane. Release is initiated by electrochemically dissolving or disrupting the barrier layer (Figure 1). Because this method is based on physical rather than chemical entrapment, the reagent to be released may be ionic, neutral, or (most interestingly) a macromolecule.

In our previous correspondence (6) we demonstrated the validity of the concept by showing that insulin and vitamin B-12 can be electroreleased from a composite membrane. The system investigated was based on a microporous alumina host membrane and a Nafion barrier layer (6). In this paper, we expand on this concept by demonstrating and evaluation other electroreleasing systems.

Furthermore we demonstrate a multidose electroreleasing membrane based on the composite membrane concept.

EXPERIMENTAL

Materials. The electrorelease membranes used in this study are fabricated on Anopore microporous filter membranes (Alltech Assoc.). Anopore is an Al_2O_3 membrane with 200 nm diameter parallel pores and a fractional pore area of 0.65. On one side of the membrane, each 200 nm diameter pore branches into several 20 nm diameter pores (Fig. 2a). Electrorelease membranes were prepared by coating the 20 nm-diameter side with the barrier layer; the 20 nm pore diameter side was used, rather than the 200 nm pore diameter side, because less material is needed to seal the 20 nm diameter pores.

To make the electrorelease membranes, the 20 nm side of the filter membrane was first sputtered with Au (Fig. 2b) and an electrical lead attached with silver epoxy. It is important to point out that this Au layer is so thin that it does not block the pores in the membrane (Fig. 2b). The membrane was then coated with the barrier layer; two different barrier layer materials were used: Ag and Nafion (Nafion is a perfluorosulfonate polymer.) (7). Ag barrier layers were prepared by electroplating Ag galvanostatically, at a current density of 1.25 mA/cm^2 (Fig. 2c) from a thiocyanate-based plating solution (8). The completed silver films are approximately 2 μm thick. Silver was used in these initial experiments because it is conveniently deposited and dissolved via the reaction $Ag^+ + e^- = Ag$.

Because of its expense and toxicity, Ag is probably not a practical barrier layer material; this is why we also investigated polymeric barrier materials in this preliminary work.

"Split" electrorelease membranes were devised in order to better control the rate of electrorelease (Fig. 3). The substrate electrodes were fabricated by masking the center of an Anopore membrane disk with a 0.35 mm-wide plastic strip. After sputtering with Au, the mask was removed, leaving two electrodes electrically isolated from each other. An electrical lead was attached to each half-electrode with silver epoxy. The silver barrier layer was then electroplated as before. The gap between the electrodes was sealed with epoxy (Fig. 3).

The Nafion barrier layers were produced by a high-temperature solution-processing procedure from an ethanol solution of Na^+-form Nafion (7). The film was cast onto the gold-sputtered Anopore which was heated to 175°C to increase the rate of evaporation of the solvent and prevent the Nafion from filling the pores. As noted above, polymeric barrier layers are undoubtedly preferable to metallic barriers.

Equipment and Procedures. The rate of electrorelease from these membranes was monitored spectrophotometrically. The cell used is shown in Fig. 4; it consists of two parts: an electrochemical cell and a dye reservoir. The electrochemical cell and dye reservoir are separated by the electrorelease membrane. The barrier layer side of the Anopore membrane faced the electrochemical cell. A platinum flag was used as the counter electrode. The electrolyte was 0.1 M

KNO_3; nitrate ion insures that the Ag^+ obtained upon dissolution of the Ag barrier layer does not precipitate. The electrolyte was degassed by purging with N_2, and stirred during the experiment with a mini-magnetic stir bar.

The cell shown in Figure 4 was placed in a Perkin-Elmer Lambda 4B UV-Visible spectrometer and the absorbance in the electrochemical cell monitored at the absorbance maximum of the electroreleased molecule. The spectrometer optical path passed through the cell approximately 1 cm in front of the electrorelease membrane. In order to open the pores in the Ag barrier layer electrorelease membrane, the silver layer was oxidized galvanostatically at a current density of 1.3 mA/cm^2 with an EGG/Princeton Applied Research Model 173 Galvanostat/ Potentiostat. To perform the electrorelease experiment with the Nafion membranes, a cathodic current of 6.2 mA/cm^2 was applied to the membrane.

The dye reservoir contained an aqueous solution of either methylene blue dye (Aldrich), $K_3Fe(CN)_6$ (Baker), or bovine insulin (Sigma). The level of the dye solution was positioned 2 to 3 cm above that of the electrolyte to increase the rate of flow of the dye solution through the electrorelease membrane. Therefore, movement of the dye through the membrane is not diffusion-controlled, but is due to a pressure-driven flow. This is proven by the fact that absorbance increased linearly with time in all of the experiments described here and in our previous paper (6). If the rate of release were diffusion-controlled, absorbance would increase with the square root of time. Furthermore, after an extensive

electrorelease period, the level of the dye solution and the receiver solution are visibly lower and higher, respectively.

RESULTS AND DISCUSSION

Silver Barrier Layer Electrorelease Membranes. Figure 5 shows plots of absorbance (at 650 nm) vs. time monitored in the electrochemical cell. Curve a is a plot of the absorbance when the silver is not oxidized. A small rise in absorbance is observed, indicating slight leakage of the dye through the membrane. This slight porosity of the silver membrane is probably caused by large silver grain size, which creates tortuous paths through the membrane. This porosity could undoubtedly be removed by thermally annealing the films; however, as noted above, we regard the Ag barrier layer membranes as test systems to prove the concept and not as practical electrorelease systems.

Curve b in Figure 5 shows the electrorelease experiment. During the first 30 minutes of curve b, the silver is being electrochemically oxidized (current on period; Fig. 5). Because the silver film acts as the barrier layer, this electrochemical oxidation opens the Anopore pores. The abrupt absorption rise at the beginning of the current on period is caused by a slight clouding of the electrolyte by silver particles. It was found that oxidation with a higher current density or without previously degassing the electrolyte increased this clouding significantly.

After 30 minutes of Ag electrolysis, the electrolysis current was turned off. The absorbance increased with time during this current off period, indicating that dye is seeping through the open

pores of the Anopore membrane. The linear increase in absorbance (correlation coefficient = 0.99) indicates that transport of dye across the membrane is not diffusion controlled. The slight sub-linearity seen at high absorbance is probably caused by deviation from Beer's Law at high dye concentration (9).

The experiment summarized in Figure 5 shows that electrorelease through these membranes occurs, and that the rate of release in this cell is constant, as is indicated by the linear increase in absorbance with time. However, this approach to electrorelease is not controllable, i. e. once the barrier layer is completely removed, the flow rate of the electroreleased reagent will be constant, and cannot be changed.

However, it is not necessary to completely dissolve the barrier layer, but merely to make it porous. The degree of porosity can be varied by controlling the amount of charge passed during the oxidation of the silver barrier layer. Thus, one could pass a small amount of charge, and open some pores, and create a slow rate of release, then pass more charge, open more pores, and obtain a faster electrorelease rate.

An experiment of this type is shown in Fig. 6. The Ag electrolysis current was switched on during the first eight minutes of this experiment (1 Coulomb of charge was passed). The current was then switched off. A linear increase in absorbance (demonstrating a constant rate of flow of the dye through the membrane) was subsequently observed. After 40 minutes the Ag electrolysis current was again switched on and an additional 0.5 C of charge was passed. The slope of the absorbance vs. time curve

increased from 2.51×10^{-3} to 7.67×10^{-3}, indicating that the rate of transport of dye across the membrane increased by a factor of three as a result of the second Ag electrolysis period. The discontinuity of the two lines (Figure 6) is due to scattering by silver particles removed during the second oxidation. By using this technique of controlling the amount of charge passed, the rate of flow through the membrane can be fine-tuned to any desired value.

Another method of modulating the rate of delivery of reagent is to divide the Anopore membrane into separate electrorelease zones. This can be done by placing separate, electrically-isolated barrier layers over several portions of the membrane. Each zone is individually addressable. A simple demonstration of this technique was performed by dividing the membrane into two semi-circular zones (Fig. 3). These zones can be independently electroreleased.

The results of an experiment of this type are shown in Fig. 7. The barrier layer over one half of the electrode was oxidized during the first "current on" period; a linear absorption (slope = 3.16×10^{-3}, $R^2 = 0.91$) increase was observed during the following 30 minute period. The barrier layer over the second half of the membrane was oxidized away during the second current on period. The resulting electrorelease rate (slope = 5.84×10^{-3}, $R^2 = 0.98$) is approximately twice the original rato indicating that flow occurs through an area of the membrane twice as large. This technique could be extended to an array of several electrodes which could be used as a multi-dose delivery system. If the solution were confined within the pores of the membrane (instead of behind the membrane as in the present system), a true multidose system would be obtained. By removing

one of the electrode barrier layers at a time, the solution under only that electrode would be electroreleased.

Nafion-coated Electrorelease Membranes. Because silver is somewhat toxic, it is undesirable to make an electroreleasing membrane from it for biomedical use. Barrier layers fabricated from inert polymers would appear to be better candidates for biomedical applications. Therefore, an electrorelease membrane was fabricated using Nafion, a chemically inert cation exchange polymer. The parent polymer of Nafion, Teflon, has excellent biocompatibility (10). Furthermore, Nafion has been used as an electrode coating for in vivo electrochemistry (11); the Nafion-coated electrode showed better in vivo electrochemical response than the analogous uncoated electrode.

In these experiments, $K_3Fe(CN)_6$ was used as the tracer dye as the Nafion membrane is permeable to cationic methylene blue. Nafion is completely impermeable to $Fe(CN)_6^{-3}$; thus, there is no unwanted leakage from these membranes. This was proven by monitoring the absorbance in the receiver solution for several hours prior to electrorelease. $Fe(CN)_6^{3-}$ could not be detected in the receiver solution.

Because Nafion is electrochemically inactive, dissolution of the film is not possible. However, it is possible to disrupt the film and tear it from the electrode by evolving gas at the electrode beneath the Nafion film To do this, a cathodic current of 10 mA was passed through the electrorelease membrane. Optical abosrption in the electrochemical cell was monitored at 450 nm.

passed through the electrorelease membrane. Optical abosrption in the electrochemical cell was monitored at 450 nm.

Figure 8 shows that the absorption, and thus, the concentration of ferricyanide, increased linearly ($R^2 = 0.99$), indicating that the Nafion film is punctured by gas evolution at the gold surface. Scanning electron microscope analyses of the Nafion after electrorelease shows hole and cracks in the film (Figure 9). These electrochemically-induced fissures are caused by gas evolution and are responsible for the electrorelease.

The ability of these Nafion-covered microporous membranes to electrorelease large biological molecules is demonstrated in Figure 10. Bovine insulin (Sigma, NW-5700) was dissolved in a pH 9.3 ammonium buffer electrolyte to which biuret reagent was added (6). The biuret reaction yielded the characteristic violet Cu(II)-insulin complex which allowed for visible spectrophotometric detection at 530 nm. When the Nafion film was disrupted as described above, electrorelease of the insulin through the membrane was observed (Fig. 10). To our knowledge, this system is the only electrorelease method capable of electroreleasing such high molecular weight species.

CONCLUSIONS

Electrorelease from membranes fabricated from a microporous membrane overlaid with a non-porous, electro-removable barrier layer has been demonstrated. These membranes contain the electroreleased reagent by physical, rather than chemical, entrapment, and therefore, electrorelese does not depend on the

chemical nature of the reagent, as in some polymeric electrorelease membranes (2,4). These new electrorelease membranes have other important advantages. First, the rate of release is not dependent on diffusion through a polymer. Thus, delivery rates are faster than for polymer-entrapped delivery systems. This is a particularly important point for biomedical applications, where high molecular weight (e.g. macromolecular systems) might be electroreleased. Diffusion of such macromolecules in polymeric membranes would be prohibitively slow. We have demonstrated the ability of this system to electrorelease large biomolecules and proteins (6).

An additional advantage is the possibility for multi-dose systems (demonstrated here). Multi-dose electrorelease systems can be fabricated by separating the substrate membrane into individually-addressable electrorelease zones, a multi-dose electrorelease device can be realized. Such a device could be used to deliver exact dosages at several specified times. A device capable of delivering many doses could easily be constructed using conventional microfabrication technology.

As pointed out by a reviewer of this paper, the electrorelease system described here bears some resemblance to iontophoresis (11,12). In iontophoresis a current is used to drive an ionic species through the skin. The skin is, then, analogous to the barrier layer in our systems. The difference, however, is that our systems operate by physically disrupting the barrier layer. As such, the current is on for only a brief time while disruption is occuring. Release then occurs for prolonged times after the current is shut off. Finally, an alternate type of multi-dose device could be constructed by coating

the Anopore membrane with an electroactive ionomer (2,3). These electrorelease polymers are capable of being switched "on" and "off" many times, providing the ability to deliver drugs or reagent repeatedly from the same membrane. Work on this electrorelease scheme is currently underway in this laboratory.

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FIGURE CAPTIONS

Figure 1 - Schematic diagram of electrorelease mechanism.

Figure 2 - Fabrication of electrorelease membrane. A. Bare 20 nm pore diameter Anopore membrane; B. Anopore membrane sputtered with Au; C. Anopore membrane with pores sealed with electroplated Ag or solution-deposited Nafion.

Figure 3 - "Split" electrorelease membranes.

Figure 4 - Experimental cell. a. Glass cells; b. Anopore membrane; c. Methylene blue or ferricyanide tracer dye; d. UV-VIS optical path; e. 0.1 M KNO_3 electrolyte; f. Pt flag counter electrode; g. Stir bar.

Figure 5 - Electrorelease of methylene blue through Ag barrier layer membrane. Absorbance monitored at 650 nm. a. No oxidizing current passed. b. Oxidizing current passed for 30 minutes during current on period.

Figure 6 - Change in electrorelease rate with currnt passed. Barrier layer partially removed during first "current on" period. Additional barrier layer removed during second "current on" period.

Figure 7 - "Split" electrode experiment. The electrorelease membrane is divided into two regions. The barrier layer over barrier layer over the second region is removed during the second "current on" period.

Figure 8 - Electrorelease of ferricyanide through a Nafion membrane.

Figure 9 - Scanning electron micrograph of fissure in Nafion film caused by gas evolution during electrorelease experiment.

Figure 10 - Electrorelease of insulin through a Nafion membrane.

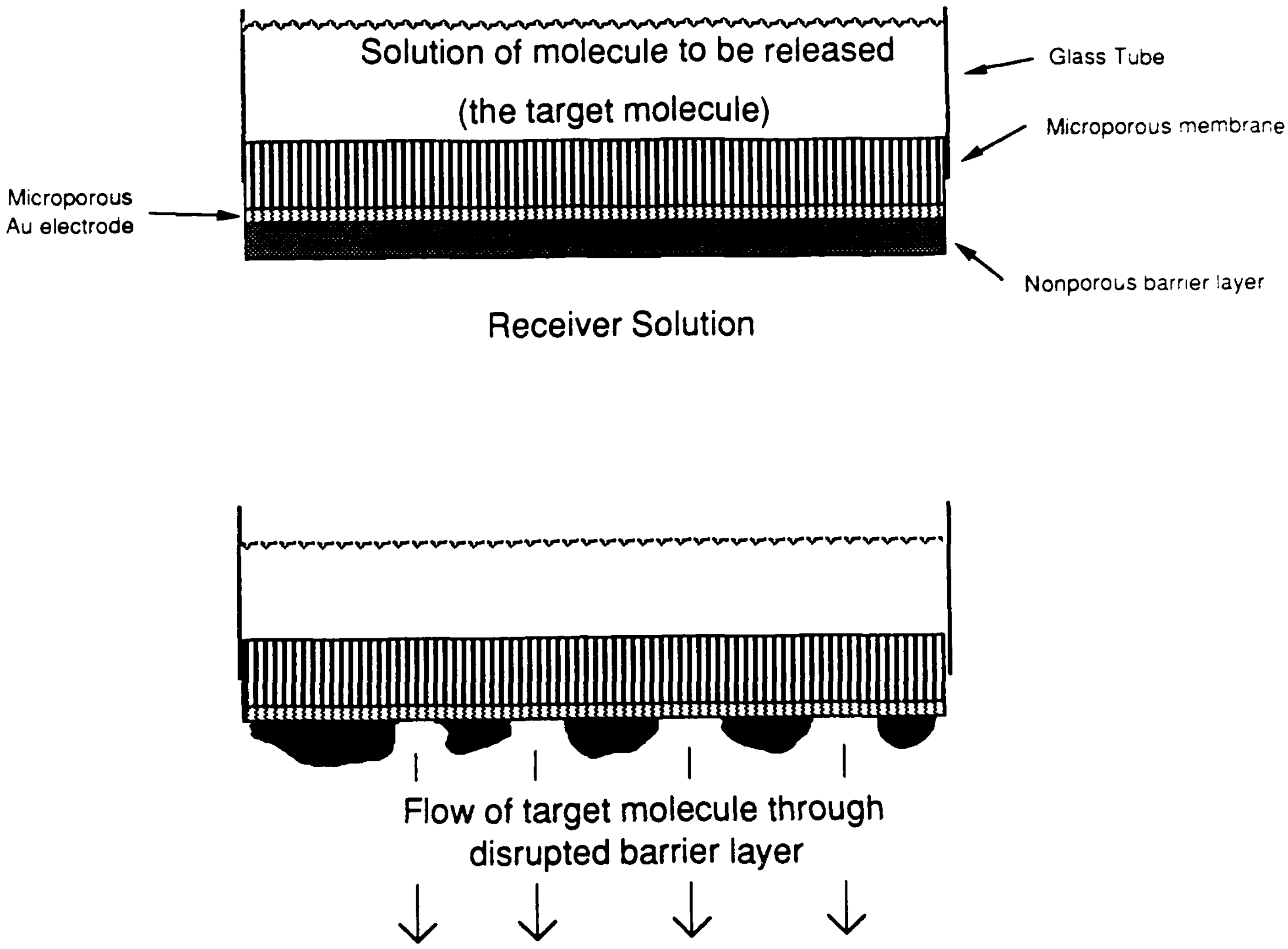

Solution of molecule to be released
(the target molecule)
Glass Tube
Microporous membrane
Microporous
Au electrode
Nonporous barrier layer
Receiver Solution
Flow of target molecule through
disrupted barrier layer

Fig. 1

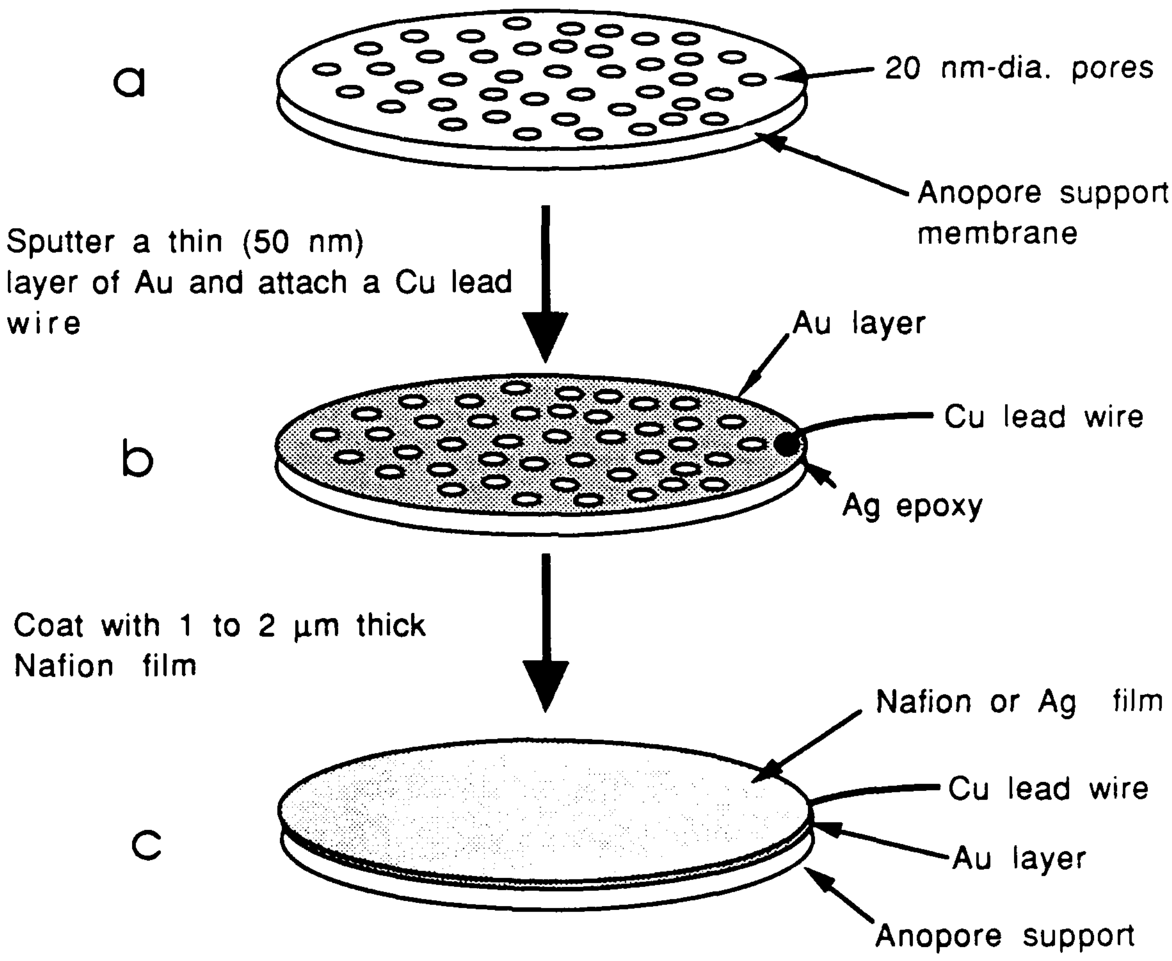

a
20 nm-dia. pores
Anopore support membrane
Sputter a thin (50 nm) layer of Au and attach a Cu lead wire
Au layer
Cu lead wire
b
Ag epoxy
Coat with 1 to 2 µm thick Nafion film
Nafion or Ag film
Cu lead wire
c
Au layer
Anopore support

Fig. 2

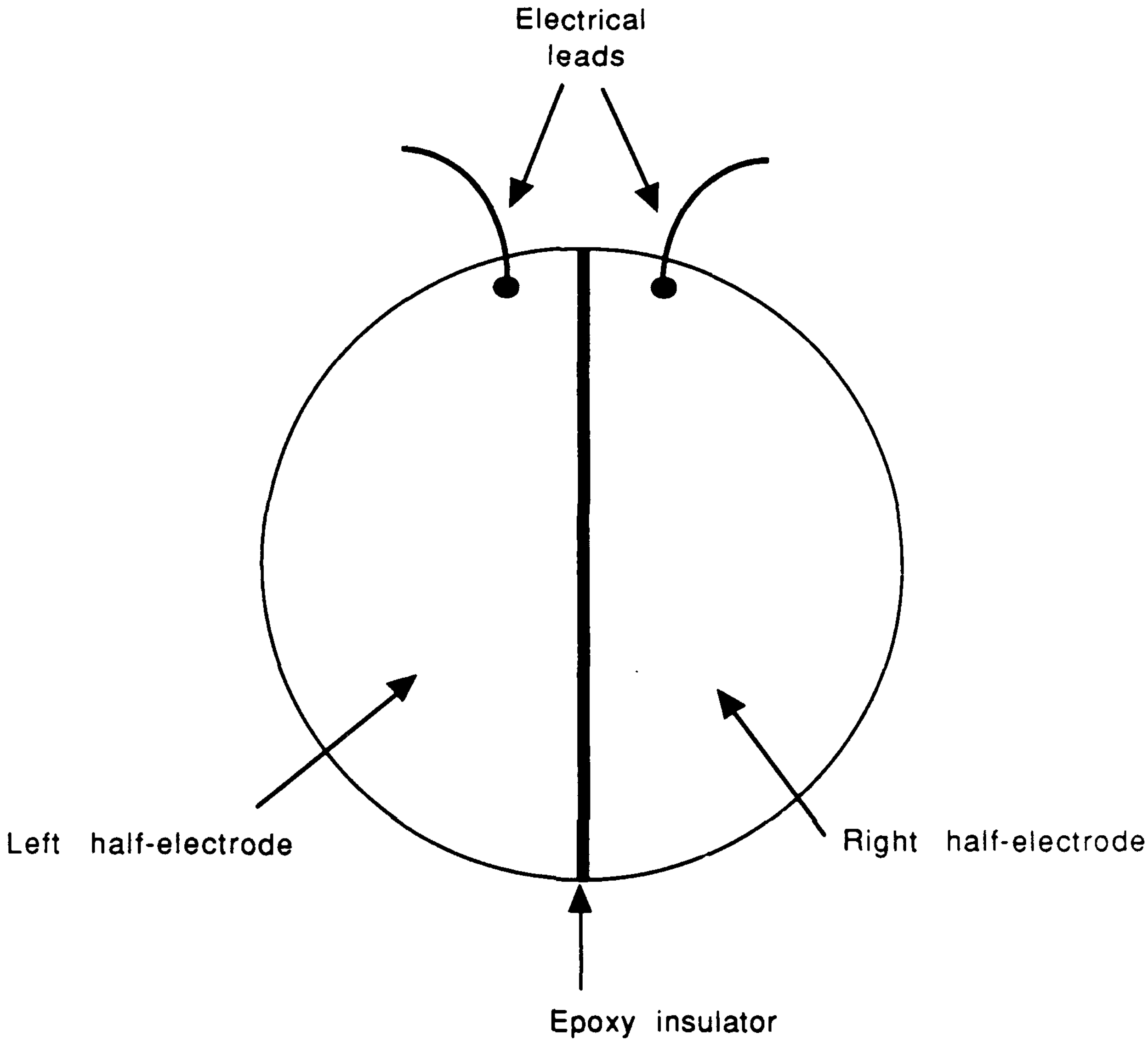

Electrical leads
Left half-electrode
Right half-electrode
Epoxy insulator

Fig. 3

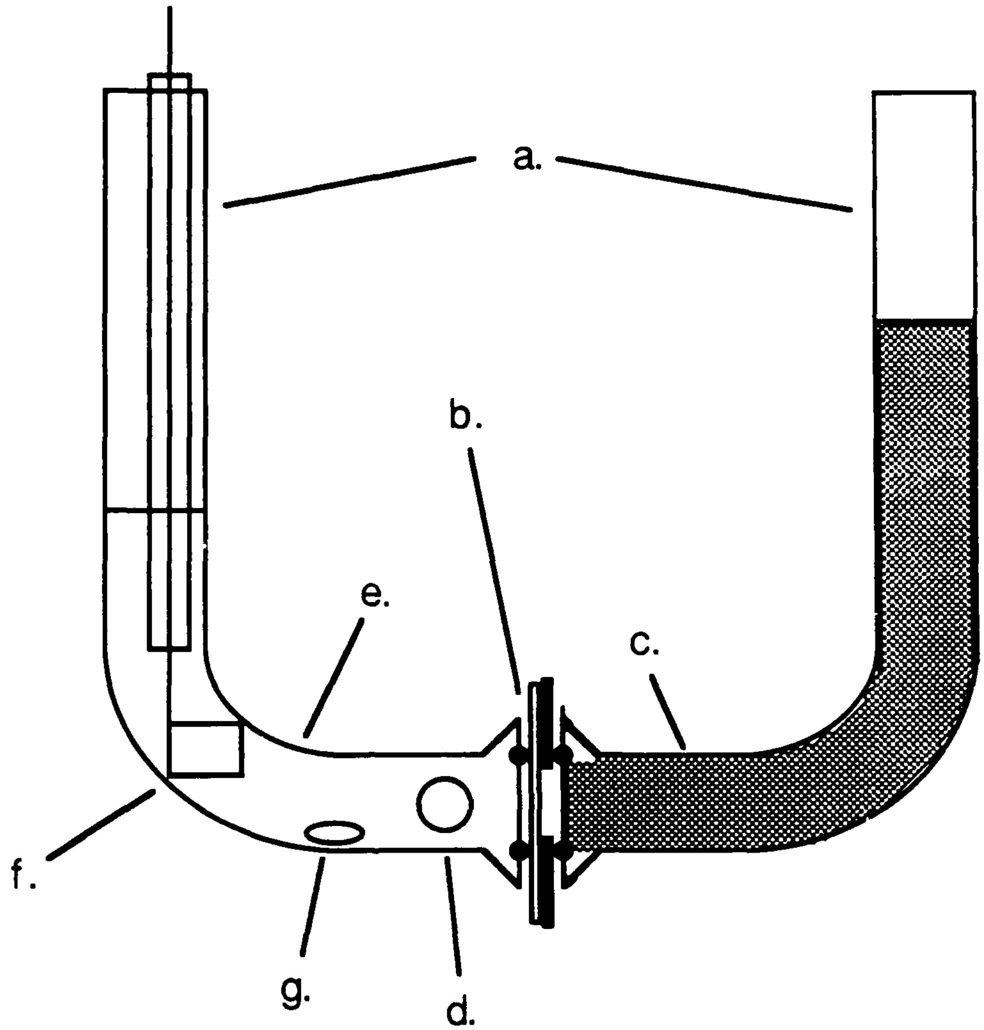


Fig. 4

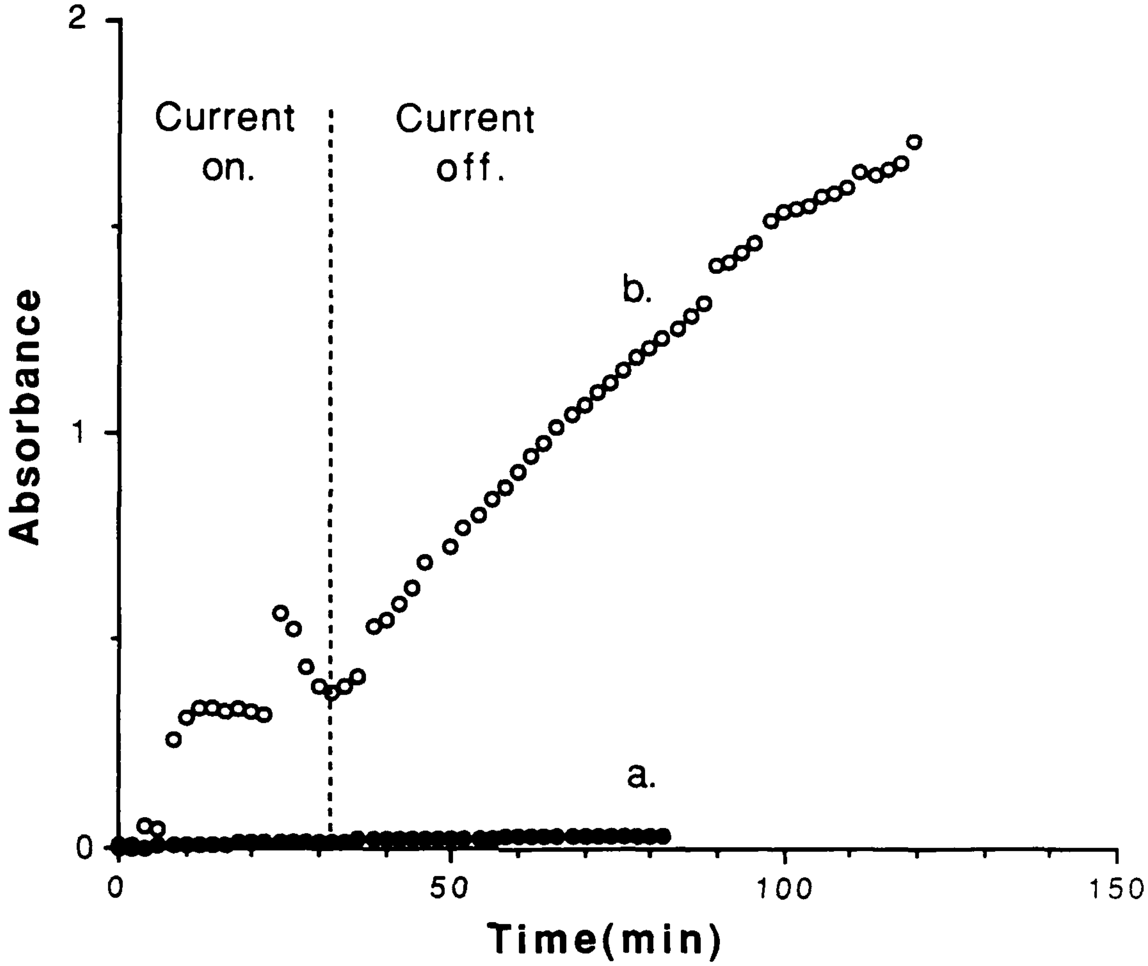

2
1
0
Absorbance
Current on.
Current off.
b.
a.
0
50
100
150
Time(min)

Fig. 5

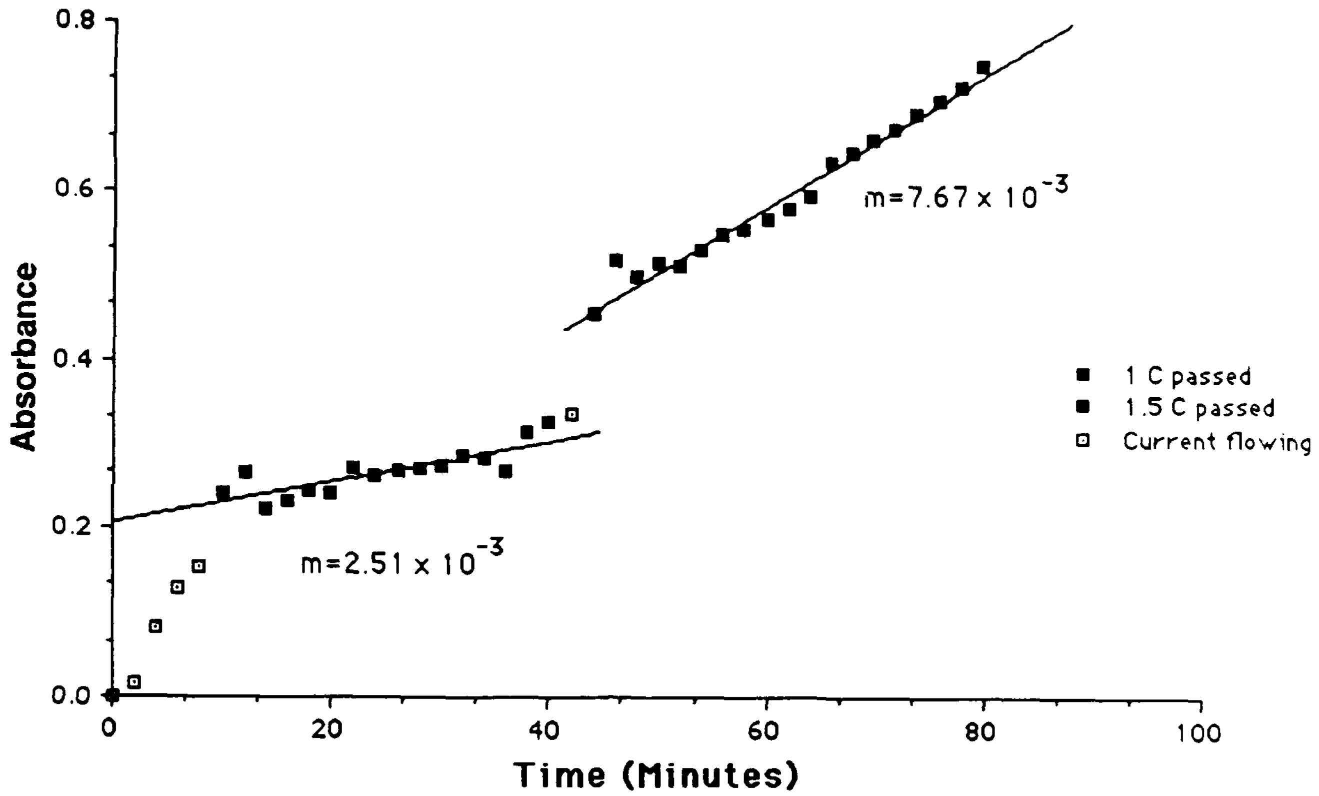

0.8
0.6
0.4
0.2
0.0
Absorbance
0
20
40
60
80
100
Time (Minutes)
m=7.67 x 10-3
m=2.51 x 10-3
1 C passed
1.5 C passed
Current flowing

Fig. 6

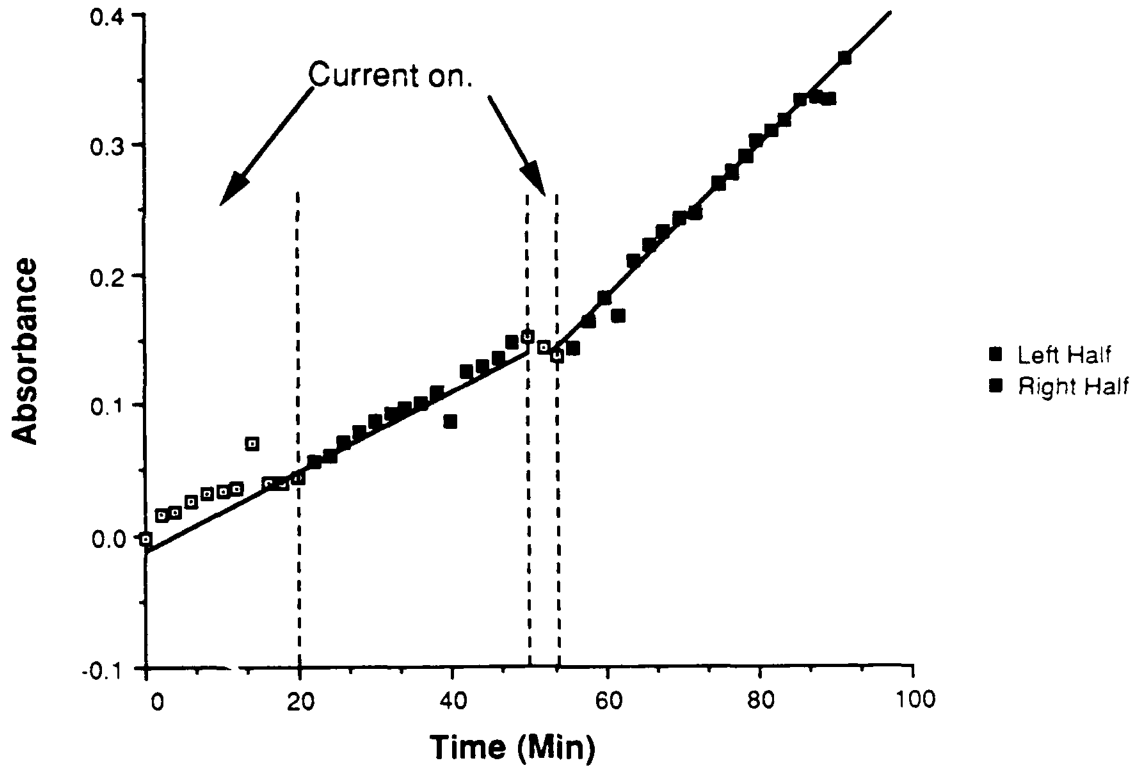
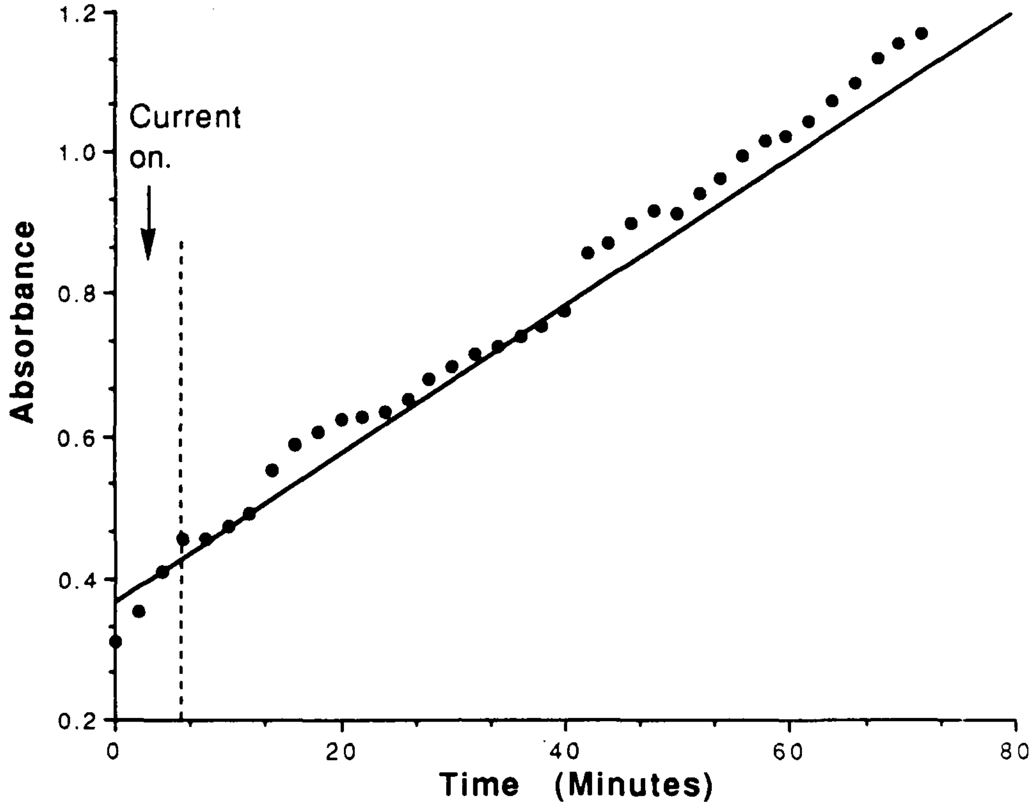
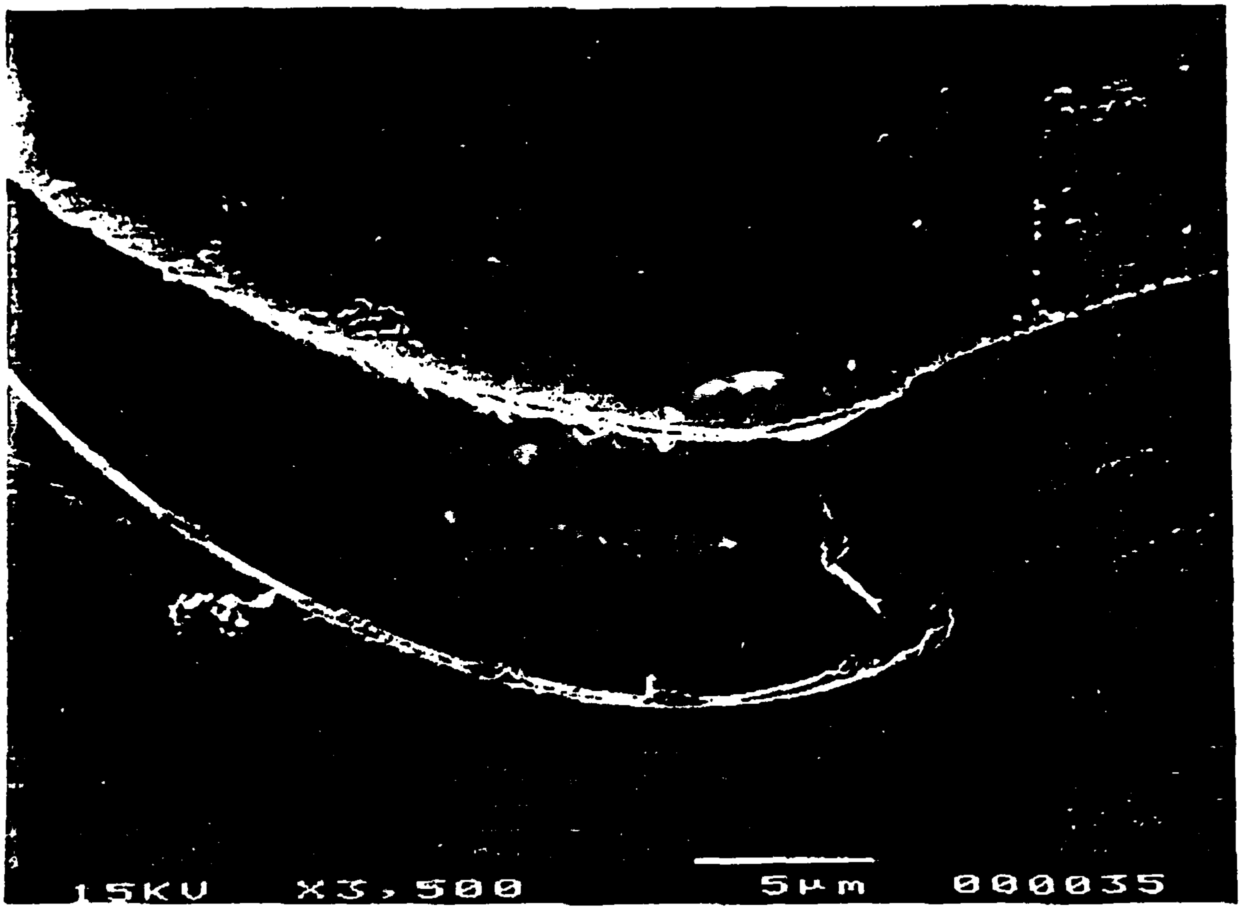

Current on.
0.4
0.3
0.2
0.1
0.0
-0.1
Absorbance
0
20
40
60
80
100
Time (Min)
Left Half
Right Half

Fig. 7



Current
on.
Absorbance
Time (Minutes)
1.2
1.0
0.8
0.6
0.4
0.2
0
20
40
60
80

Fig. 8



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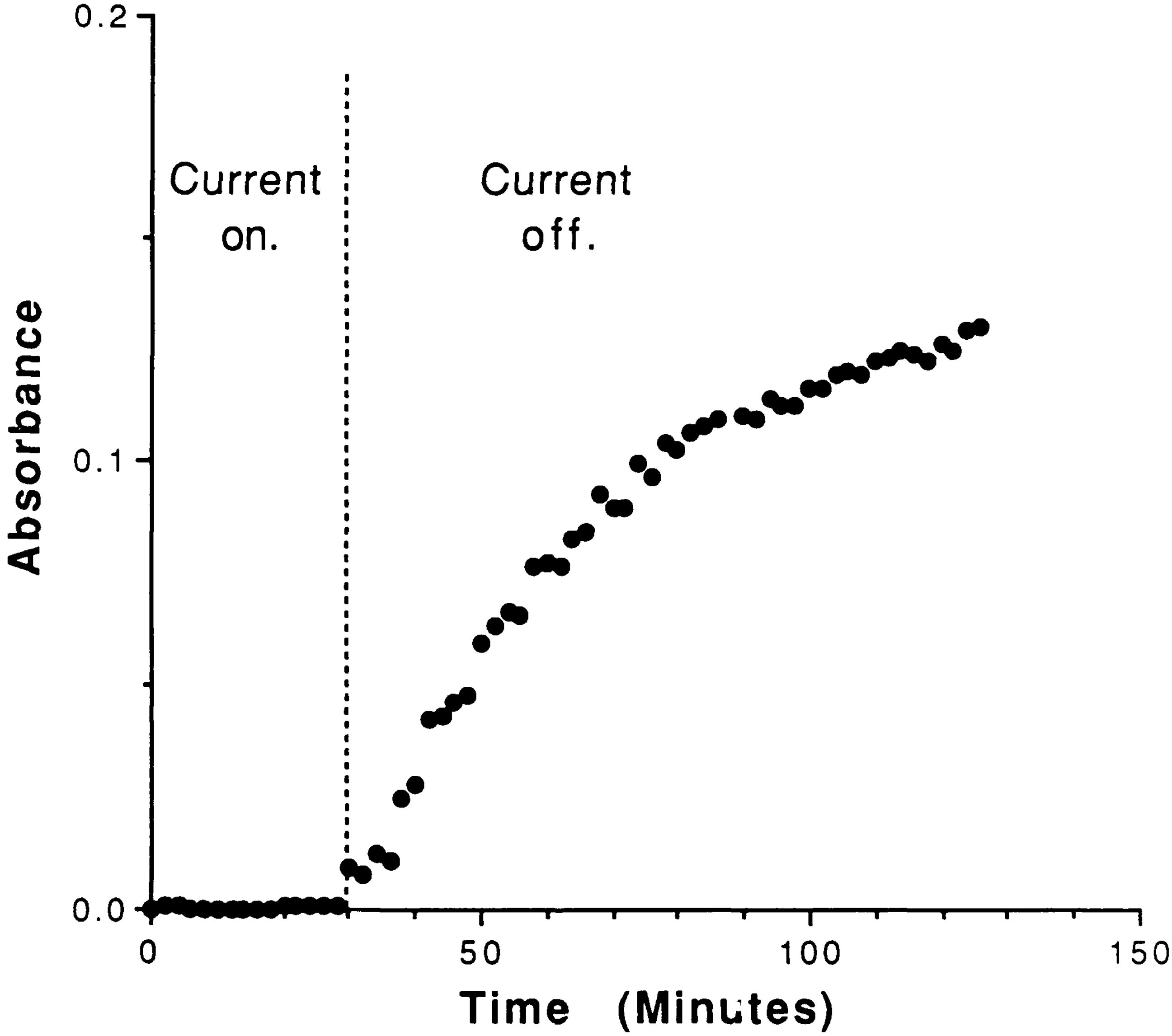
0.2
0.1
0.0
Absorbance
Current on.
Current off.
0
50
100
150
Time (Minutes)

Fig 10